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THE USE OF FLUORESCCEIN ANGIOGRAPHY
TO STUDY OXYGEN TOXICITY

by

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Naval Medical Research and Development Command
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PROBLEM

To test whether fluorescein angiography can be used as a technique for monitoring an individual's response to abnormally high levels of oxygen.

FINDINGS

Monkeys subjected to 100% oxygen for periods of several days developed maculopathy which could be seen in fluorescein angiograms; the damage was reversible since all evidence of pathology disappeared within two weeks following the exposures.

APPLICATION

Angiograms can be used as an early warning system, for individuals subjected to increased oxygen levels (divers, patients undergoing oxygen therapy), since they can be removed from the increased oxygen before permanent damage occurs.

ADMINISTRATIVE INFORMATION

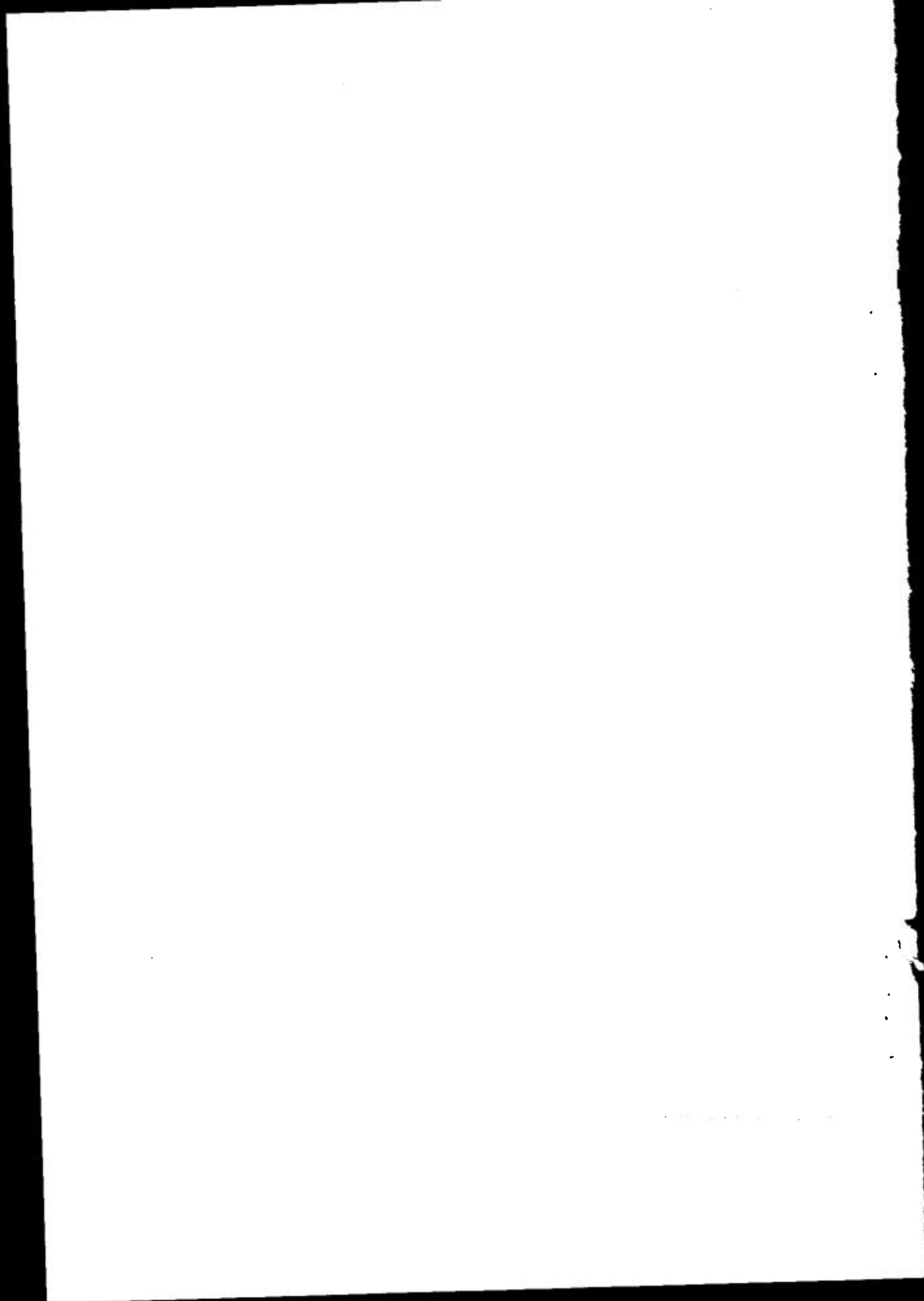
This investigation was conducted as part of Nav Med Rsch & Dev Command Rsch Work Unit # MR041.01.01-0130. The present report is Number 5 on this Work Unit. It was submitted for review on 28 Feb 1976, approved for publication on 3 Mar '76 and designated as NavSubMedRschLab Report No. 833.

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The Use of Fluorescein Angiography to Study Oxygen Toxicity*

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The Use of Fluorescein Angiography to Study Oxygen Toxicity*

This study was designed to test whether oxygen toxicity results in retinal damage that could be documented by the technique of fluorescein angiography. Three squirrel monkeys lived in 100% oxygen environments for periods ranging from 50 to 117 hours. Two of the monkeys incurred damage as evidenced by early multifocal leakage and subsequent accumulation of dye in the retina. All monkeys survived the exposures and were followed for several weeks thereafter. The pathology appeared to be reversible since later angiograms were either normal, or approaching normality, when the animals were sacrificed. Subsequent histopathologic examination revealed no abnormalities.

Oxygen toxicity can be a grave concern whenever man breathes oxygen at partial pressures greater than normal. Since this occurs routinely in air diving and in the therapeutic use of oxygen, it is essential to monitor man's response to assure that exposure is discontinued before incapacitating or even lethal

consequences ensue. Monitoring is usually based upon well known signs and symptoms, such as respiratory distress or subjective reports of the shrinkage of the visual field.

Another, more objective, measure of oxygen toxicity is suggested by investigations of the effects of hyperbaric oxygen in the eyes of dogs. Beehler and associates^{1,2} and Yanoff and co-workers³ have shown that severe retinal detachments occur in dogs after breathing 90% to 100% oxygen for 2 to 3 days. Yanoff's explanation was that the oxygen induced changes in the permeability of the choroidal blood vessels allowing fluid to leak through into the subretinal space. A similar explanation was given for a case of retinal detachment from an entirely different cause, toxemia of pregnancy⁴; in this case, fluorescein

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angiograms showed multiple leaks of dye from the choroid.

The suggestion then is that the pulmonary edema and exudates of the lung, a common result of oxygen toxicity, have their counterparts in the leakages and hemorrhages of the choroid blood vessels of the eye; furthermore, these ocular abnormalities can be photographed using the technique of fluorescein angiography and this measure used as an indication of the progression of the poisoning. We decided to test this hypothesis by taking fluorescein angiograms of squirrel monkeys who were subjected to 100% oxygen for periods of 2 to 5 days. The choice of squirrel monkey was based upon the fact that, of all the species studied thus far, subhuman primates resemble man most closely in their pulmonary response to oxygen poisoning.⁵ Furthermore, among subhuman primates, squirrel monkeys are the most resistant to oxygen.⁶

Method

The subjects were 3 adult squirrel monkeys of about 7 years of age. Prior to photography, the monkeys were injected with 0.5 ml ketamine and their pupils were dilated with 1% Mydriacyl. Ten minutes later, with the monkey in position in front of the camera and the retina in focus in the view finder, 10% Fluorescite was injected into a vein. Fundus photographs were taken immediately, at a rate of about one per second, for the next 20 seconds; a 5 minute rest was followed by other pictures. The photographs were taken on Tri-X film with a Zeiss camera equipped for fluorescein photography.

A complete set of fluorescein angiograms was taken of each monkey before oxygen exposure and several sets were made on each during the 2 weeks following exposure. During the exposure period, angiograms were made daily;

this necessitated removing the monkeys from the oxygen for approximately one half hour.

For the oxygen exposure, the monkeys lived in a Plexiglas cage 24 x 26 x 15 inches, which contained a perch, food, and water. One hundred percent oxygen was fed to the cage; the outlet was fitted to a vacuum pump which discharged through a flow meter, and hence to a Servomex O₂ Analyzer and an IR 315 CO₂ Analyzer. Sodasorb was added to the cage to aid in CO₂ control. Levels of O₂ and CO₂ were monitored hourly. The average oxygen level was $98.9 \pm 0.8\%$; the range was 97% to 100%. CO₂ average was $0.094 \pm 0.03\%$; the absolute range lay between 0.02% and 0.155%.

The total duration of oxygen exposure varied for each monkey. The first monkey, who lived by himself in the cage, was maintained for a total of 50 hours on oxygen. He showed no signs of oxygen toxicity and served mainly as a trial subject for the various procedures. The second and third monkeys were placed in the cage together; the first pictures were taken after 50 hours of exposure. Monkey #2 was returned to his normal environment after 75 hours of oxygen, when it became apparent that he had signs of oxygen toxicity appearing in the photographs. The third monkey remained in the oxygen for a total of 117 hours. The durations of air exposures during these periods have been subtracted from the totals. Monkey #1 was removed from the cage 3 times; monkey #2, twice; and monkey #3, 4 times, for picture taking. All monkeys survived the exposures.

Results

Monkey #1

This monkey survived 50 hours of exposure to 100% oxygen with no signs of toxicity, either retinal or otherwise.

MONKEY NO. 1

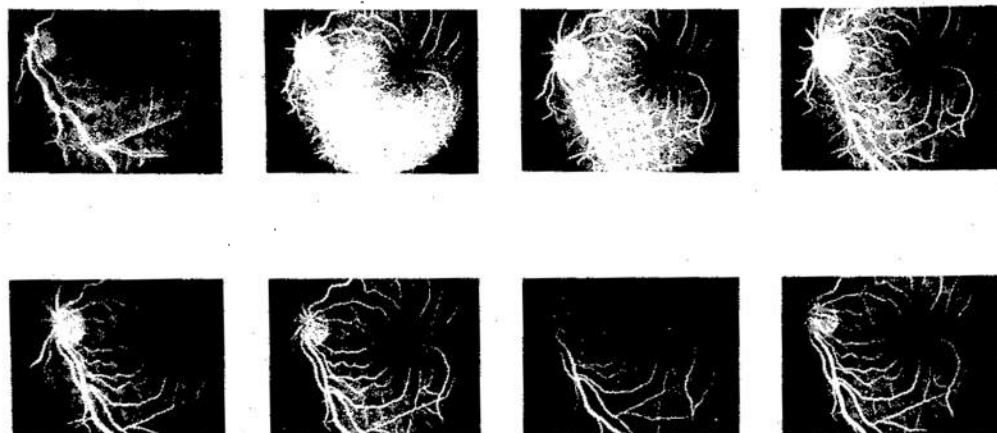


FIG 1. Fluorescein angiograms of the right eye of monkey following 50 hours exposure to 100% oxygen.

Fluorescein angiograms of the right eye were normal; examples are given in Figure 1. Ophthalmoscopic examination revealed no abnormalities nor did the histopathologic investigations.

Monkey #2

This monkey survived 75 hours of exposure to 100% oxygen without any signs of oxygen poisoning. However, at this time, during the routine fluorescein angiograms, diffuse early subretinal leakages were seen in both eyes, the dye occupying only the macular areas. The photographs on this day were unfortunately very fuzzy, as can be seen in the pictures in Figure 2. Nevertheless, the location and pattern of the dye accumulation could still be ascertained in both the early and late photographs.

After this series of photographs, the monkey was removed from the oxygen and his progress followed for 15 days, after which he was sacrificed for histopathologic study. Fluorescein angiograms taken both 5 and 8 days following exposure were completely normal, as is shown in Figure 2. Fundus photographs, taken in color, on the same post-exposure days showed bilateral disciform macular elevations which contained yel-

lowish subretinal exudates. Ophthalmoscopic examination, 11 days after oxygen exposure, revealed maculae with a slightly yellow cast but an otherwise normal appearance. Histopathic examination likewise showed no signs of pathology, either in the eyes or in the lungs.

Monkey #3

This monkey was removed from oxygen after 117 hours; at this time there were no leakages apparent in the angiograms of the right eye nor any visible signs of leakage in the left eye. However, during the first post-exposure control, 3 days later, early multifocal leakage appeared with subsequent accumulation of dye. Evidence of damage was found in all post-control runs, 6, 16, and 19 days after exposure, although the affected area was more circumscribed on the latter 2 angiograms. Figure 3 shows sample photographs taken 5 minutes after fluorescein injection, of the same area of the right eye, in the pre-exposure pictures and in angiograms 3, 6 and 19 days after exposure to 117 hours of oxygen.

Ophthalmoscopic examination revealed bilateral disciform macular

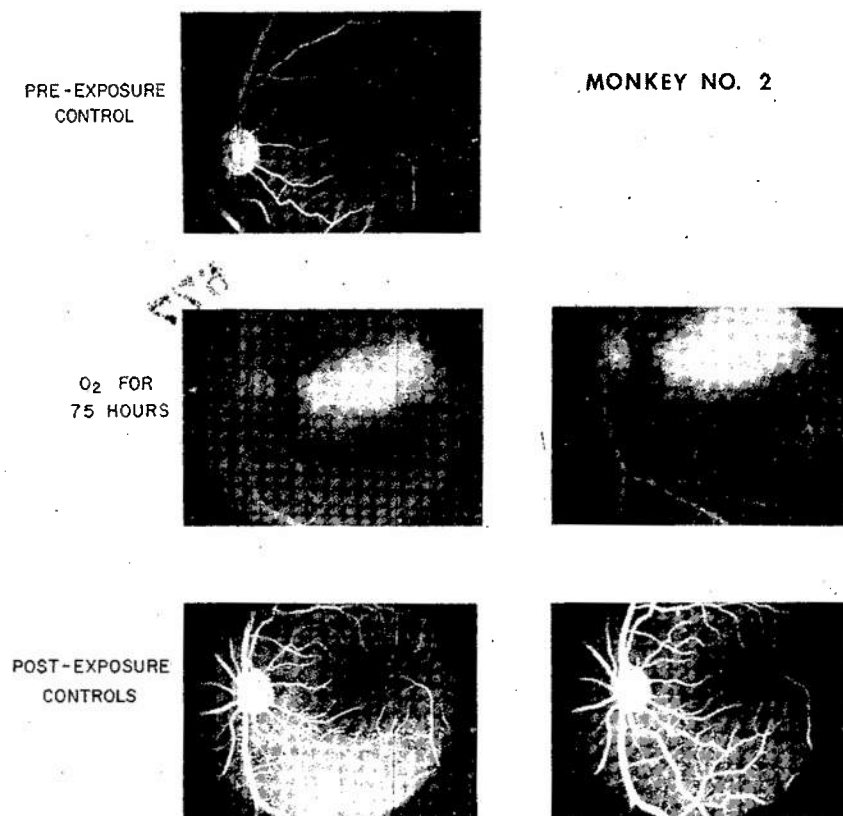


FIG 2. Sample fluorescein angiograms of the right eye of monkey prior to oxygen exposure, after 75 hours of exposure to 100% oxygen, and 5 days following exposure.

elevations which contained yellowish subretinal exudates. The animal was sacrificed after a period of 20 days in a normal environment. Histopathologic examination showed no abnormalities ascribable to oxygen in either the lungs or eyes.

Discussion

These trials, while admittedly exploratory, did yield results substantial enough to indicate that fluorescein angiography holds promise for the study of oxygen toxicity. Two of the 3 monkeys showed fluorescein leakages which were documented in the photographs; when returned to a normal environment, one monkey recovered completely and the other had improved considerably before

being sacrificed. This suggests that the technique may have useful applications in monitoring health and in understanding the mechanisms of oxygen toxicity. While considerable knowledge has resulted from studies using tissue cultures, the detailed mechanism is still unknown⁷; a technique allowing study of the toxic effects and of the agents that modify them could be extremely useful.

The results must be considered exploratory only, since so many unforeseen problems arose and unanswered questions remain. It was difficult to inject the tiny veins of squirrel monkeys with the fluorescein dye and once used, a given vein had to be abandoned and another

MONKEY NO. 3

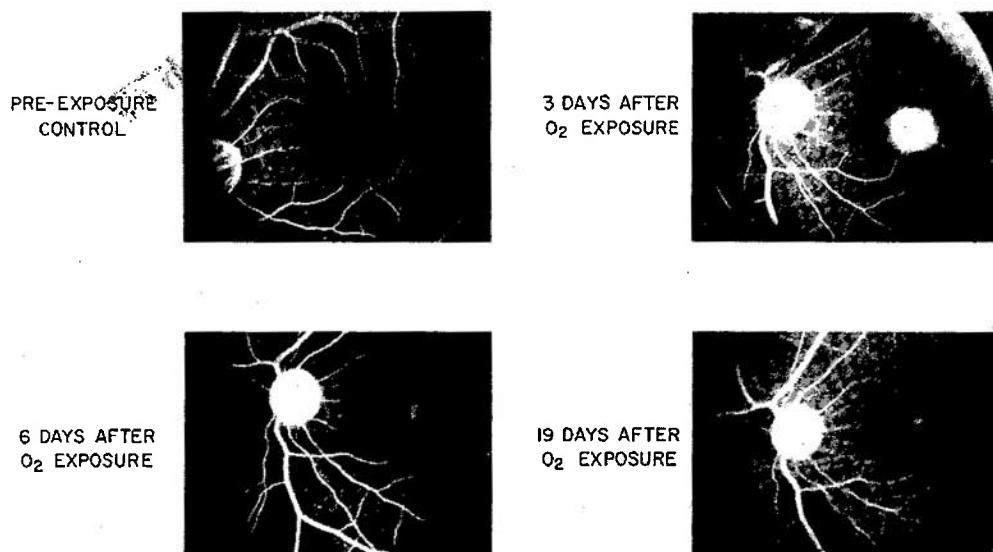


FIG 3. Sample fluorescein angiograms of the right eye of monkey prior to exposure and at various times following exposure to 100% oxygen for 117 hours.

one employed for the next set of pictures; the result was the monkeys eventually ran out of usable veins. Focusing the light on the eye proved difficult since the cornea tended to dry during the series of pictures resulting in a filmy haze which obscured the view. Differentiation of the choroidal and retinal phases of the fluorescein angiograms necessitates pictures within the first few seconds after injection and this was often not achieved. Nonetheless, the problems were gradually overcome with experience and ingenuity and the feasibility of future studies is proved.

Unanswered questions concern the site of the lesions, the degree of individual differences, and the permanence of the damage. The site may be the choroid vessels, as suggested by Yanoff and colleagues.³ The lesions produced did closely resemble the serous disciform macular detachments described by Gass⁸ in some cases of collagen disease and severe hypertension. Gass also was

of the opinion that the macular lesions were secondary to choroidal vascular disease. An alternate explanation is that the leakage occurred because of damage to the retinal pigment epithelium.

The response to the hyperbaric oxygen differed among the monkeys, making interpretation difficult. Nonetheless, individual differences in tolerance to hyperbaric oxygen are well documented in the literature^{5,9}; for example, in 4 squirrel monkeys studied by Robinson and his group,⁶ 3 died on days 8, 9, and 13 while the fourth survived a 14-day exposure. It seems likely, therefore, that the differing response of monkeys #2 and #3 simply represents normal individual differences in O₂ tolerance.

Finally, the damage incurred under these conditions appears to be reversible; one monkey incurred early damage, in 75 hours; he also recovered early, within 5 days following exposure. The second monkey was more tolerant to the oxygen originally, with no evidence of damage

for 117 hours. However, he sustained a delayed damage which proved to be more resistant to recovery. This too, appeared to be reversible, as evidenced by the improving angiograms and the lack of abnormalities in the histopathologic examination. Nonetheless, a large group of animals on a controlled schedule of exposure will be required to decide the incidence of damage and of recovery, and to determine if there is a relationship between them.

Acknowledgment

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can be seen in the photographs as it flows through the retinal vascular system. The suggestion is that the pulmonary edema and exudates of the lung, a common result of oxygen toxicity, have their counterpart in leakages and hemorrhages of the blood vessels of the eye and that these leakages can be seen in the fluorescein pictures.

This suggestion was tested by taking fluorescein angiograms of squirrel monkeys who were subjected to 100% oxygen for periods of 2 to 5 days. The monkeys did incur retinal damage which could be seen as the accumulation of dye in the retina. The monkeys survived the exposure and returned to normal within two weeks after the oxygen exposure. The technique thus appears promising for monitoring exposure to oxygen poisoning since individuals can be removed from the oxygen before permanent damage occurs.